

```

gtcctctcct actactcatt gccaaagatcc tttaagcaca cccagcgccc cggggagccg 3361
gaggaggggtg cccggcatca gcacctccgc ctttccacta gctctgggtcg ccttctctat 3421
gctgcaaacag cagatgatcc ttctctttcc accagcagcg acagcctggg tgggggatac 3481
tgccggggcta ggctggagcc cagccttcca catccccacc atcaggttct gcagcccat 3541
5 ctgcctcgaa aggtggacac agctgctcag accaatagcc gcctggcccgc gccacgagg 3601
ctgtccctgg gggatcccaa ggcagcacc ttacctcggg cccgagagca gcagcagcag 3661
cagcagccct tgetgcaccc tccagagccc aagagcccgg gggaatatgt caatatggaa 3721
tttgggagtg atcagtctgg ctacttgctt ggcccgggtg ctttccacag ctcaccttct 3781
gtcagggtgc catcccagct ccagccagct cccagagagg aagagactgg cactgaggag 3841
10 tacatgaaga tggacctggg gccgggcccgg agggcagcct ggcaggagag cactggggtc 3901
gagatgggca gactggggccc tgcacctccc ggggctgcta gcatttgca gcctaccg 3961
gcagtggcca gcagccgggg tgactacatg accatgcaga tgagttgtcc ccgtcagagc 4021
tacgtggaca cctgcgcagc tgcccctgta agctatgctg acatgcgaac aggcattgct 4081
gcagaggagg tgagcctgcc cagggccacc atggctgctg cctcctcatc ctcagcagcc 4141
15 tctgcttccc cgactggggc tcaaggggca gcagagctgg ctgcccactc gtccctgctg 4201
ggggggccac aaggacctgg gggcatgagc gccttcaccc ggtgaacct cagtcctaac 4261
cgcaaccaga gtgccaagt gatccgtgca gaccacaag ggtgccggcg gaggcatagc 4321
tccgagactt tctcctcaac acccagtgcc acccggttg gcaacacagt gccctttgga 4381
gcggggggcag cagtaggggg cggtggcggt agcagcagca gcagcgagg tgtgaaacgc 4441
20 cacagctctg cttcctttga gaatgtgtgg ctgaggcctg gggagcttg gggagcccc 4501
aaggagccag ccaaaactgt tggggctgct ggggggttgg agaattggtc taactacata 4561
gacctggatt tgggtcaagg cttcaaacag tgccctcagg agtgacccc tgaaccgcag 4621
cctccccac cccaccccc tcatcaaccc ctgggcagcg gtgagagcag ctccaccgc 4681
cgctcaagtg aggatttaag cgcctatgcc agcatcagtt tccagaagca gccagaggac 4741
25 cgtcagtagc tcaactggac atcacagcag aatgaagacc taaatgacct cagcaaatcc 4801
tcttctaact catgggtacc cagactctaa atatttcatg attcacaact aggacctcat 4861
atcttctca tcagtatag gtacgatgca tccatttcag tttgtttact ttatccaatc 4921
ctcaggattt cattgactga actgcacgtt ctatatttg ccaagcgaaa aaaaaaatg 4981
cactgtgaca ccagaataat gagtctgcat aaacttcac ttcaacctta aggacttagc 5041
30 tggccacagt gagctgatgt gccaccacc gtgtcatgag agaattgggt tactctcaat 5101
gcattttcaa gatactttc atctgctgct gaaactgtgt acgacaaaagc atcattgtaa 5161
attatttcat acaaaaactgt tcacgttggg tggagagagt attaaatatt taacataggt 5221
tttgatttat atgtgtaatt ttttaaatga aaatgtaact tttcttacag cacatctttt 5281
ttttggatgt gggatggagg tatacaatgt tctgttgtaa agagtggagc aaatgcttaa 5341
35 aacaaggctt aaaagagttag aatagggtat gatccttgtt ttaagattgt aattcagaaa 5401
acataatata agaactatag tgccatagat ggttctcaat tgtatagtta tatttgctga 5461
tactatctct tgtcatataa acctgatgtt gacctgagtt ccttataaga attaacttta 5521
atthttgtatt ttttctgta agacaatagg ccatgttaat taaactgaag aaggatatat 5581
40 ttggctgggt gttttcaaat gtcagcttaa aattggtaat tgaatggaag caaaattata 5641
agaagaggaa attaaagtct tccattgcat gtattgtaaa cagaaggaga tgggtgattc 5701
cttcaattca aaagctctct ttggaatgaa caatgtgggc gtttgtaaat tctggaaatg 5761
tctttctatt cataataaac tagatactgt tgatcttita aaaaaaaaaa aaaaaaaaaa 5821
aaaaaaaa (SEQ ID NO:6; GENBANK Accession No. NM 005544)

```

The double mutation of tyrosine 897 and 1180 was  
 constructed by replacement of 3'-sequences coding 897F by  
 the same region of 1180F using restriction enzymes NheI and  
 EcoRI, and this construct was called 897F1180F or  $\Delta$ Grb2  $\Delta$ Syp.  
 The expression plasmids were under control of a CMV promoter  
 (hIRS-1-wt,  $\Delta$ Grb2,  $\Delta$ Syp,  $\Delta$ Grb2,  $\Delta$ Syp and pBK-CMV (mock) and  
 linearized at the 3'-end of poly A signal sequences by MluI  
 restriction enzymes followed by purification. A similar  
 approach was used to change the tyrosine residue to